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MAR 13 1930

BULLETIN

OF THE

JOHNS HOPKINS HOSPITAL

(THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL)

(SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY)

Vol. XLVI, No. 3 MARCH, 1930

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Entered as second-class matter at the Baltimore, Md., Postoffice. Acceptance for mailing at special rate of postage provided for in Section 1103, act of October 3, 1917. Authorized on July 3, 1918.

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A PHYTOPHARMACOLOGICAL STUDY OF ECLAMPSIA[1]

DAVID I. MACHT AND JOSEPH R. LOSEE[2]

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INTRODUCTION

One of the authors (M), for quite a number of years, has been carrying on general pharmacological studies on the comparative reactions of animal and plant protoplasm to various drugs and poisons. Such studies soon made it evident that living plant tissues, or living plant protoplasm, may react very differently to certain drugs, toxins, or chemicals from the way in which living animal tissue, or living animal protoplasm, reacts to the same substances (1). Frequently a drug of plant origin is found to be more poisonous for animals than for plants and, vice versa, toxins of animal origin are found to be very much more poisonous for living plant protoplasm than for living animal protoplasm. Such studies on the effects of drugs and poisons on living plant tissues have been carried on by Macht on the growth of roots and stems of various seedlings, on the germination of seeds, on the transpiration of water through the stomata of leaves, etc., and to the new field of pharmacology thus developed the term phytopharmacology has been applied. The most useful method for quantitative phytopharmacological studies was found to be the study of the growth of the straight, well-defined roots of the seedlings of *Lupinus albus*, an European plant belonging to the lupine or bean family. By such methods, Macht and Lubin have been able to demonstrate the presence of a toxin in the blood, saliva, milk, sweat, tears and other secretions of menstruating women (2). These studies, which are of historical interest, led to a discovery of more practical importance, namely, the first experimental demonstration, by phytopharmacological methods, of the presence of a toxin in the blood of pernicious anemia, which

[1] Received for publication, October 5, 1929.

[2] Died February 5, 1929.

is not present in the blood of patients suffering from secondary anemias, carcinoma, leukemia, and other blood conditions (3). Again, Macht and Pels have discovered, by phytopharmacological methods, for the first time, the presence of a toxin in the blood of pemphigus (4), while other medical applications of plant physiological methods were recently reported by Macht before the XIIIth International Congress of Physiology in Boston (5).

As early as 1923, through the courtesy of Dr. E. D. Plass, Macht had an opportunity of securing a specimen of blood serum from a case of severe eclampsia. This specimen was tested by the usual methods on plant growth and was found to be absolutely non-toxic, that is, it gave the same reaction as normal blood serum. This suggested the desirability of a further investigation or study of eclamptic blood in order to determine whether the new phytopharmacological methods would reveal the presence of a toxin in this grave disease, which has for many years been regarded as a toxemia. Through the kindness of Dr. H. J. Stander of the Obstetrical Department, Johns Hopkins Hospital, a number of specimens from eclamptics were secured from time to time and others were obtained through the courtesy of Dr. J. Greenhill, of the Chicago Lying-In Hospital. The examination of those specimens rendered a complete investigation on the subject so desirable that in December, 1927, Macht, with the kind collaboration of Dr. J. R. Losee, of the New York Lying-In Hospital, began a more extensive study of the subject. In the present communication, the results obtained from a study of some thirty cases of eclampsia and toxemias of pregnancy are reported.

METHODS OF STUDY

Macht's method of determining the phytotoxic index of blood serum has already been described elsewhere. It consists, briefly, in measuring quantitatively the increment in the length of the straight, single, well-defined roots of seedlings of *Lupinus albus*, a hardy plant especially useful for this purpose. The average length of ten or more such seedlings is measured and the seedlings are then immersed in a suitable plant physiological solution, containing all the elements necessary for perfect growth of plants, and allowed to grow for a period of twenty-four hours in the dark, after which the roots are again meas-

ured and the average increment determined. The figure thus obtained is the standard of comparison for other series of seedlings immersed in the same kind of nutrient solution, but containing in addition a given percentage of a blood serum or drug, the effect of which one desires to ascertain. The ratio of growth thus obtained, as compared with normal controls, is taken for the index of growth. In this way, a series of seedlings immersed in normal nutrient solution, plus one per cent of normal human blood serum, is found to give an index of 70 to 75 per cent. A third series of seedlings, grown simultaneously in the same kind of nutrient solution but containing a *pathological* blood serum, the serum of pernicious anemia, is found to give an index of growth or toxicity, as compared with the original normal standard, of 50 per cent or even less. In this way, with a sufficient number of seedlings on hand, it is possible to examine a large number of blood sera simultaneously and under exactly the same conditions of temperature, weather, light, etc., and thus determine the relative toxicity of the various samples.

The authors wish to emphasize that, in order to carry out the phytopharmacological test carefully, a great deal of training is required not only in ordinary pharmacological methods but also in the laboratory methods pertaining to plant physiology. If a beginner expects to succeed in such work in the first attempt, he will more than likely be disappointed; indeed, he may not be able even to germinate the seeds, much less to secure uniform seedlings suitable for testing. The seeds employed by Macht and his coworkers are those of a large variety of *Lupinus albus*. They must not be too old or worm-eaten. They are soaked in tap water for twenty-four hours to insure their complete swelling, and are then planted in a suitable germinating medium. The most suitable germinating medium for this purpose is finely ground sphagnum moss. It is of vital importance that, after such moss has been ground to a very fine powder, it contains an amount of moisture suitable for germination of the particular seed. Here is one of the first stumbling-blocks the beginner may encounter. If too much or too little water is present in the moss, the seedlings will give a stunted growth or will suffer from malnutrition. Experiments made to determine the water content in sphagnum moss most suitable for germination showed that it must contain about 75 or 80 per cent of its

weight of water. In preparing the sphagnum for germination, fresh moss must usually be rinsed with hot water a number of times in order to dissolve and remove acid matter, which may interfere with the proper growth of the plant. After planting the swollen seeds in a properly prepared sphagnum medium, the vessel containing them is placed in the dark at room temperature and the germination is allowed to go on. When the seedlings attain a length of from 20 to 30 millimeters or more, depending on temperature and other conditions, they are ready for testing. Several rows of hard-glass tubes are set up in a perpendicular position, each row consisting usually of ten tubes. In the first row, ten seedlings are immersed in the normal nutrient solution, consisting of equal parts of distilled water and Shive solution, described elsewhere. In the succeeding rows a similar nutrient solution is employed, plus one per cent of the blood serum to be examined. The seedlings are carefully measured with a millimeter rule and immersed in the solutions which have been thus prepared. The whole outfit is then placed in an incubator or other suitable place in the dark. It is essential that the growth of the seedlings should be carried on at a proper temperature, 20° to 22°C. being the most suitable. When kept at a higher temperature, growth may be so greatly stimulated as to mask small differences between the various sets of seedlings (6).

MATERIAL EXAMINED

Twenty-eight different cases of eclampsia and related toxemias of pregnancy are included in the present study. One of these was obtained through the courtesy of Dr. Plass; five were obtained from Dr. Greenhill of Chicago; eight from Dr. Stander in Baltimore; and the rest from the Lying-In Hospital, New York. In addition to these twenty-eight cases, two other pathological cases, diagnosed as toxemia of pregnancy, were also studied and gave somewhat anomalous results, which will be discussed separately. The results obtained from a study of the 28 cases are shown in Table I. It will be noted that the average index of growth of the seedlings obtained is 75 per cent. This indicates that the toxicity of eclamptic blood is the same as that of normal human blood serum or, if anything, slightly less. Two specimens, studied in addition to the 28 mentioned above, gave somewhat different results. Both of these were sent with a diagnosis of toxemia

of pregnancy without convulsive seizures. One of these gave an index of 47 per cent and the other of 55 per cent. In connection with these two cases, it is impossible to exclude contamination of the specimens, so that the low figures obtained may have been due to such contamination. Nevertheless, these two exceptions to the rule are noted in this place because they may have been special cases which were included under the general term of toxemia, but which were probably different from the frank cases of eclampsia and toxemia of pregnancy usually encountered. The terms toxemia of pregnancy and eclampsia are

TABLE I

CASE NUMBER	INDEX OF GROWTH	CASE NUMBER	INDEX OF GROWTH
	per cent		*per cent*
I (from Dr. P.)	79	XVI (from Dr. L.)	78
II (from Dr. S.)	72	XVII (from Dr. L.)	72
III (from Dr. S.)	76	XVIII (from Dr. L.)	72
IV (from Dr. S.)	75	XIX (from Dr. L.)	75
V (from Dr. S.)	74	XX (from Dr. L.)	71
VI (from Dr. S.)	72	XXI (from Dr. L.)	75
VII (from Dr. S.)	73	XXII (from Dr. L.)	72
VIII (from Dr. S.)	70	XXIII (from Dr. L.)	72
IX (from Dr. S.)	88	XXIV (from Dr. L.)	80
X (from Dr. G.)	70	XXV (from Dr. L.)	76
XI (from Dr. G.)	76	XXVI (from Dr. L.)	70
XII (from Dr. G.)	78	XXVII (from Dr. L.)	85
XIII (from Dr. G.)	71	XXVIII (from Dr. L.)	85
XIV (from Dr. G.)	70		—
XV (from Dr. L.)	73	Average.....	75

more or less general and may occasionally include other pathological conditions.

DESCRIPTION OF CASES

In order to convey an idea of the variety of cases, from which the blood was examined by phytopharmacological methods, the following brief résumé of some of the histories is given.

A. *Anna M.:* 34 years old; para V; 6½ months pregnant. Admitted to the hospital with pain in the right hypochondrium at 2.45 p.m. Blood pressure, 200/150. Almost solid albumin. Convulsion two hours after admission. Phle-

botomy—600 cc. blood. Morphine and atropine administered. She died at 3.30 a.m. the following morning. A typical case of eclampsia convulsions. Pain in the right hypochondrium undoubtedly due to subcapsular hemorrhage of the liver. On admission to the hospital, the pulse was 70; four hours later, 160; and, between convulsions, went down to 90, which would make one consider the possibility of a cerebral hemorrhage. Phytotoxic index, 72 per cent.

B. *Julia K.:* 23 years old; para I; at term. Admitted to the hospital with a urine showing albumin + and hyaline and granular casts, and some oedema of the extremities. Blood pressure, 122/68. No previous history. Wassermann (++). Probably a mild toxemia without convulsions. Phytotoxic index, 70 per cent.

C. *Jennie D.:* 28 years old; para V. Typical case of eclampsia seven years previously with first baby. Symptoms of toxemia with second baby. Third and fourth pregnancies not accompanied by any complications. Admitted to the hospital on account of a marked trace of albumin with hyaline casts. Blood pressure, 102/58. Eight months pregnant. The patient does not look sick. Rather a good case on account of previous history. To be considered as a mild case of toxemia. Phytotoxic index, 76 per cent.

D. *Pauline R.:* 34 years old; para V. Stout woman weighing 215 pounds. Previous pregnancies all normal. At present albumin +; no casts. Blood pressure, 180/98. Not very sick at the present time. Phytotoxic index, 80 per cent.

E. *Rose S.:* 42 years old; para VIII. Large stout woman weighing 200 pounds. Blood pressure, 198/80. Almost solid albumin with an occasional granular cast. No oedema; no changes in the fundus of the eye. About at term, complaining of indefinite pain in the chest. No trouble with previous babies. Phytotoxic index, 70 per cent.

F. *Beckie M.:* 35 years old; para I; at full term. Blood pressure, 178/120. Oedema, albumin, casts and headaches. Phytotoxic index, 73 per cent.

G. *Anna R.:* 23 years old; at full term. Blood pressure, 155/100. Marked trace of albumin; granular and hyaline casts. Had one convulsion lasting two minutes after forty hours in labor. Phytotoxic index, 78 per cent.

H. *Emily W.:* 37 years old; 9 months pregnant. Blood pressure, 120/100. Oedema. Three convulsions from 7 a.m. to 10 a.m. Blood taken at 5 p.m. Phytotoxic index, 71 per cent.

I. *Ethel S.:* 31 years old; para II; at full term. Gradual rise in blood pressure from January 31, 1928, 135/100, to March 22, when it was 170/100. Delivered March 23. Two convulsions after delivery. Phytotoxic index, 75 per cent.

J. *Lillian F.:* 22 years old; para I; 7½ months pregnant. Blood pressure, 190/130. Marked trace of albumin with hyaline and granular casts. Oedema. Eye-grounds normal. Phytotoxic index, 71 per cent.

K. *Mrs. Mou.:* Para I; at term. Three convulsions and coma. Blood pressure, 110/60. Albumin and casts. Oedema. Phytotoxic index, 85 per cent.

L. *Mrs. S. P.:* 36 years old; para II; at term. Delivered at 5 a.m. First convulsion at 9.30 a.m.; second convulsion at 9.50 a.m.; third convulsion later in the day. Showed albumin and casts. Blood pressure, 190/110. Blood taken during second convulsion. Phytotoxic index, 85 per cent.

DISCUSSION

The etiology of eclampsia has never been absolutely established. By far the most prevalent conception of this disease, or symptom-complex, has for many years been that of a toxemia, but experimental proof of such toxemia is lacking. The difficulties in regard to the subject of etiology have been succinctly set forth in the recent article by Zondek, whose paper should be familiar to all those interested in the subject (7). In the last few years a new conception concerning the etiology of eclampsia has been advanced, more particularly by Zangemeister (8). This author, as a result of extensive clinical observations and experimentation, has come to the conclusion that eclamptic seizures are due primarily to the water-logged or hydropic condition of the tissues and his views are therefore not in favor of a direct toxicosis as the cause of the convulsions. The findings of the present writers obviously do not support the toxemic conception of eclampsia but tend rather to favor the conception of Zangemeister. More accurately speaking, the present experiments speak against the existence in the blood of eclamptics of a poisonous substance, which is toxic for the living seedling of *Lupinus albus*. This does not, of course, exclude the possibility of other abnormal products of metabolism being present in the blood which are not poisonous for plant protoplasm. Thus, for instance, the recent views concerning the rôle played by histamine in eclampsia, advanced by Hofbauer (9), cannot be considered as discordant with the findings of the present authors, for one of the writers (M) has found that both histamine and pituitary extract, even in considerable concentration (1:10,000), produce no appreciable inhibition in the growth of *Lupinus albus* seedlings. It is interesting to note that recently, in a short paper, Hüssy (10) has also attempted to show biologically, by means of phytopharmacological methods, the presence of a toxic substance in eclamptic blood. The procedure employed by that author, however, was so crude that the results obtained are inconclusive. Another remarkable feature of the present investigation is the fact, noted by one of the authors (M) on many occasions, that various nephropathic conditions and cases of hypertension exert apparently no toxic effect on the growth of *Lupinus albus*.

SUMMARY

1. Phytopharmacological examination of blood sera from a series of eclamptic patients and other severe toxemias of pregnancy fails to reveal any toxic effect on the growth of seedlings of *Lupinus albus*.

2. These findings speak against the presence of a toxin in the blood of eclampsia, but do not disprove the presence of other abnormal bodies which are not poisonous for plant protoplasm.

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PHYTOPHARMACOLOGICAL STUDY OF PUERPERAL BLOODS[1]

DAVID I. MACHT AND HARRIET LEACH

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INTRODUCTION

One of us (Macht), in collaboration with Dr. Dorothy Lubin in an extensive study published elsewhere, (1) has shown that by means of phytopharmacological methods developed by Macht and his collaborators (2) a toxin could be demonstrated in the blood serum and various secretions of women just before the onset of and during the menstrual periods. In another paper, appearing previously, Macht and Losee (3), using similar methods, reported the phytopharmacological findings obtained from a study of a series of cases of eclampsia and toxemias of pregnancy. In connection with these investigations, it was deemed interesting to inquire into the phytopharmacological reaction of blood specimens obtained from women after puerperium. Such an investigation was rendered possible through the kind cooperation of Professor J. Whitridge Williams of the Johns Hopkins University, Department of Obstetrics. The method of study need not be recapitulated in full in this place because it has been described in detail elsewhere (4). The phytotoxic properties of postpuerperal blood were studied by making a one-per-cent solution of the sera obtained from various patients in a suitable, plant-physiological, nutrient solution and noting the effect produced by such sera on the elongation or growth of the single, straight, well-defined roots of young seedlings of *Lupinus albus*.

MATERIAL EXAMINED

The material examined was serum obtained from blood specimens drawn from "come-back" patients of the Lying-In Department of the

[1] Received for publication, October 5, 1929.

Women's Clinic, Johns Hopkins Hospital. In this clinic, as a routine procedure, every maternity case is required to return for a complete examination six weeks after the birth of the child. The authors endeavoured to obtain specimens from as many of such "come-back" patients in good health as possible.

A total number of 223 blood specimens from as many patients was examined. When obtaining the specimens, a note was made as to the general condition of the patient, the date of the birth, the sex of the child, and, especially, the time of return for examination to the clinic after the birth of the offspring. Of the 223 women thus examined, a total of 196 were examined phytopharmacologically. In all these cases, the patient returned for examination after a lapse of from six to seven weeks from the time of delivery. The other cases were discarded as being too long after delivery or on account of the exhibition of various complications. In addition to blood from these 196 cases, a large number of normal control specimens were obtained from the clinical laboratories of Johns Hopkins Hospital and from outside. The readings obtained with non-puerperal normal blood sera and from the obstetrical cases described above are given below.

RESULTS OBTAINED

The phytotoxic index for the fifty specimens of normal blood serum from women was 74 per cent. Another series of about 30 normal blood sera, studied in connection with the present research, gave an average phytotoxic index of 72 per cent. In addition to these, a series of 30 positive Wassermann specimens, which were used as controls, gave an average index of 79 per cent.

The 196 postpuerperal cases, studied six weeks after delivery, were divided into two groups, according to the sex of the children borne. Ninety-seven cases of postpuerperal blood after male births were examined and ninety-nine cases of postpuerperal blood after female births. This division was deemed worth while because certain chemical differences between male and female blood have been described in recent years by the Russian investigator, Manoiloff, and by others (5). It was found that the average index of growth in the male series was 82 per cent. In the case of the females, the average index of growth was 78 per cent. In computing these averages, in order to render the

data as accurate as possible, statistical methods were employed. The variability of the individual readings obtained was studied by determining the figures for standard deviation and coefficient of variation. According to the directions of Garrett (6), in order to determine the standard deviation, the following formula was used,

$$\sigma = \sqrt{\frac{\Sigma D^2}{N}}$$

in which ΣD^2 is the sum of the squares of the mean or average deviations, taken from the average of the distribution, and N represents the number of cases. The coefficient of variation, V, is computed according to the following formula,

$$V = \frac{100\,\sigma}{\text{Average reading}}$$

In case of the 97 male births, the standard deviation was 1.113 and the coefficient of variation, 1.35. In case of the 99 female births, the standard variation was 1.31 and the coefficient of variation, 1.41. It will be noted that the readings obtained after the male births were slightly higher than those obtained after female births. Whether this difference of 4 per cent is of any significance, it is impossible to say, although the figure 4 per cent is considerably greater than the standard of deviation for any of the readings. It will be further noted that the phytotoxic indices obtained from both male and female births were definitely higher than those obtained with normal sera from non-obstetrical cases.

EFFECT OF AGING ON BLOOD SPECIMENS

Among the most interesting and unexpected observations made in connection with the present investigation were the findings obtained with the various obstetrical blood sera after a later or second examination. The routine procedure followed by the authors was to examine the specimens obtained from the clinic either on the same day or on the following day. The balance of the serum, however, was put in the refrigerator and a second or later test was made about a week later.

From previous experiences, Macht and his co-workers have learned that, as a rule, a specimen of blood serum which has been kept in the

ice-box gives the same phytotoxic reading as the original examination or, sometimes, a slightly higher one. In other words, these sera seem to lose their toxicity slightly on standing. Growth of bacteria and slight putrefaction do not increase the toxicity of blood serum for *Lupinus albus* seedlings. Indeed, a number of bacterial toxins were examined and were found by one of the authors to affect the growth of seedlings only very slightly or not at all. On examining all the postpuerperal sera a week after drawing the blood, a remarkable difference was noted in this respect. Even though the specimens were well preserved in the refrigerator, all these sera were found to have become much more toxic. Thus, in case of the male specimens, instead of the average index of growth of 82 per cent obtained on the first reading, the second reading gave 63 per cent, while in case of the females, in place of the average first reading of 78 per cent, the second reading gave an average of 62 per cent. For convenience of study, all the data obtained are exhibited in the following table.

DISCUSSION

A study of the subjoined table reveals certain interesting facts. It will be noted that the average figure for normal non-obstetrical blood sera was found to be 72 per cent on the first reading. A second reading made a week later gave 74 per cent, thus showing no increase in toxicity. The average normal reading of positive Wassermann specimens was found to be, on first reading, 79 per cent; on second reading, 81 per cent, again revealing no increased toxicity on standing. In contrast to these findings, the obstetrical bloods revealed a marked increase in toxicity in all the bloods.

The study described above, while of purely academic interest, is suggestive from a practical point of view, especially if one is inclined to regard bacteria as microscopic vegetable organisms. Bacteria are generally considered as being more closely related to plants than to animals and, for this reason, small plants have been utilized as indicators, to a certain extent, of the antiseptic properties of various substances. Thus, Dreser (7) employed yeast for such a purpose and, in this country, Peterson (8) has also used yeast suspensions for the evaluation of various antiseptics. Many authors have studied the

bactericidal power of blood during pregnancy and the puerperium, and its relationship to the development and course of puerperal infections. One of the most interesting contributions on this subject in relation to the present investigation is the extensive study published by Miller and Whitaker in the British Medical Journal (9). These authors have studied the bactericidal properties of pregnant individuals throughout the course of pregnancy, labor, and puerperium and, quoting from their findings: "There appears to be a gradual increase in bactericidal activity of blood as pregnancy advances, which reaches its maximum about full term. After labor, however, there is a rapid decline in bactericidal power, although, still later, a slight increase

TABLE I

KIND OF BLOOD SERUM	PHYTOTOXIC INDEX FIRST READING (WITHIN 24 HOURS AFTER BEING DRAWN)	NUMBER OF CASES	PHYTOTOXIC INDEX SECOND READING (ONE WEEK LATER; FROM REFRIGERATOR)	NUMBER OF CASES
	per cent		*per cent*	
Normal controls	72	100	74	50
Puerperal after male births	82 $\sigma = 1.113$ $V = 1.35$	97	63 $\sigma = 1.174$ $V = 1.89$	83
Puerperal after female births	78 $\sigma = 1.31$ $V = 1.41$	99	62 $\sigma = 1.113$ $V = 1.79$	78

to about the normal level, and even somewhat higher, was noted." The figures obtained with fresh puerperal sera by the present writers, which are much higher than those of normal control specimens, may signify that such blood is lacking in certain normal toxins or protective bodies. This is especially interesting in view of the high readings obtained by the writers with sera of patients suffering from syphilis and tuberculosis, to be published elsewhere (10). The remarkable increase in toxicity of the postpuerperal sera, noted on standing for a week after being drawn, cannot be ascribed to putrefactive changes because the authors have made numerous studies in regard to the effect of putrefaction on normal blood serum, as well as on the effect of marked changes in the hydrogen-ion concentration of blood sera on

growth of *Lupinus albus* seedlings. As a rule, ordinary bacterial putrefaction of normal blood serum renders its toxicity, if anything, less than normal. The changes noted in the postpuerperal sera, therefore, must be ascribed to some specific biochemical alterations in those sera which are not ordinarily found in normal controls. The differences obtained in the readings between the maternal bloods after male births and maternal bloods after female births is not very great. Nevertheless, inasmuch as this difference of 4 per cent was considerably greater than the deviations of the average readings, it may indicate possibly a real difference in the composition of the two kinds of blood. This is of greater significance in view of the fact that in dealing with normal sera of a very large number of patients, in connection with all the previous phytopharmacological researches, Macht and his collaborators have not found any difference between normal male and normal female (non-pregnant) bloods.

SUMMARY

1. A series of 196 postpuerperal blood sera, obtained six weeks after delivery, were examined by phytopharmacological methods and compared with various normal and certain pathological controls.

2. It was found that the phytotoxic index of postpuerperal sera was, in general, higher than that of normal human female blood serum.

3. This relatively non-toxic reaction of postpuerperal sera, however. becomes very much more toxic on standing in the refrigerator, and such an increase in toxicity cannot be ascribed to ordinary putrefactive changes, thus pointing to some special difference in the biochemical composition of puerperal blood, as compared with normal blood.

4. Certain slight differences in readings after male births, as compared with those obtained after female births, have also been noted and described.

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I. THE EFFECTS OF MERCUROCHROME AND MILK PROTEIN UPON ANAPHYLAXIS[1,2,3]

LAY MARTIN, M.D., AND JUSTINA H. HILL, M.S.

(From the James Buchanan Brady Urological Clinic and the Gastro-Intestinal Division of the Department of Medicine of The Johns Hopkins University)

We wish to limit this discussion entirely to the consideration of non-specific substances in their effects upon anaphylaxis in guinea-pigs. The results which we have obtained probably represent a temporary desensitization. In reviewing the articles which have been published on the subject of non-specific desensitization, we are impressed by: (1) the enormous amount of material which has been used in the effort to desensitize guinea-pigs; (2) the great amount of very badly controlled work which has been produced; and (3), the uncritical attitude of general reviewers, who have accepted the conclusions of various writers without making any serious effort to examine the protocols and materials which were used and from which the facts were deduced. Up to the present time we have been able to find over 95 different drugs which have been used for non-specific desensitization. A review of this subject will be published elsewhere but a few of the substances used may be briefly mentioned here.

Heparin has been used by several investigators and various opinions are held as to its effectiveness in preventing shock. Mineral waters from certain spas have been tried extensively by the French. There are many reports on heterologous sera and serum fractions. There are two widely divergent opinions upon the results obtained from peptone. Kopaczewski (1) and his co-workers have done considerable work with sodium bicarbonate and sodium carbonate. Calcium chloride is a much discussed material and has been tried extensively.

[1] Presented in part before the Society for the Study of Asthma and Allied Conditions, May 4, 1929.

[2] Received for publication July 10, 1929.

[3] This work was made possible by a grant from the Hugh H. Young Research Fund. We wish to thank Dr. Young for his interest.

The results of sodium chloride administration are also the basis for much polemic.

PROBLEMS

For some time we have been interested in the action of two substances which we have seen used rather extensively. One of these, mercurochrome, when used intravenously, offers many complex problems which are seemingly unrelated to the direct germicidal action of the drug when used locally in strong concentrations. One of us (2) has recently shown that the addition of mercurochrome in non-germicidal amounts to normal serum greatly strengthens the ability of mice to protect themselves after inoculation with many times the lethal dose of pneumococci. Work by one of us (3), done upon peptic ulcers, and their treatment with intramuscular injections of milk, has presented complexities similar to those encountered in the study of mercurochrome administered intravenously. It would seem from the study of these results, that these substances may act in the same way. Be this hypothesis right or wrong, we concluded that a study of their action upon anaphylaxis was one aspect of the general problem worthy of investigation. Moreover, there are certain clinical observations which suggest that these substances may cause desensitization, or a temporary inhibition of sensitization. A sudden change is seen in certain septicemias treated with mercurochrome intravenously, and in infections in which we believe there could be no possible direct germicidal action of the drug upon bacteria present in the primary focus. In peptic ulcers, of course, we do not know that the ulceration is a result of an allergic reaction, but such a condition does not seem to us outside the realm of possibility. In this case, also, the results often come so quickly, that it may be possible that we are desensitizing a body already sensitized to some unknown antigen. We, therefore, outlined experiments which we hoped would show us whether these two substances would increase, decrease, or leave unaltered the reaction of the sensitized animal.

METHOD

A. Experimental animals. Our medium of experiment was always the guinea-pig. The animals varied in weight from 190 to 440 grams.

Sensitization was carried out with subcutaneous injection of 1.0 cc. of a 1:1000 dilution of impalpable powder of egg-albumen manufactured by Merck. The incubation period was two weeks and during this time various methods of desensitization were tried as follows:

TABLE I

Typical protocol of an experiment

Set XIV—guinea-pigs which received milk protein only and the guinea-pigs which were used as controls.

PIG NUMBER	WEIGHT	SENSITIZING DOSE 0.1 PER CENT ALBUMEN		DESENSITIZING DOSE MILK PROTEIN		SHOCKING DOSE 0.1 PER CENT ALBUMEN		RESULTS
		Date	Amount	Date	Amount	Date	Amount	
				Test pigs				
	grams	*1928*	*cc.*	*1928*	*cc.*	*1928*	*cc.*	
1	280	October 17	1.0	October 24 October 26 October 30	1.0 1.0 1.0	October 31	1.0	No immediate shock, recovery
2	315	October 17	1.0	October 24 October 26 October 30	1.0 1.0 1.0	October 31	1.0	Delayed shock, recovery
3	260	October 17	1.0	October 24 October 26 October 30	1.0 1.0 1.0	October 31	1.0	Sudden death in 3–5 minutes
4	330	October 17	1.0	October 24 October 26 October 30	1.0 1.0 1.0	October 31	1.0	Sudden death in 3–5 minutes
5	345	October 17	1.0	October 24 October 26 October 30	1.0 1.0 1.0	October 31	1.0	No symptoms, recovery
6	250	October 17	1.0	October 24 October 26 October 30	1.0 1.0 1.0	October 31	1.0	No symptoms, recovery
7	260	October 17	1.0	October 24 October 26 October 30	1.0 1.0 1.0	October 31	1.0	Sudden death in 3–5 minutes

TABLE I—*Concluded*

PIG NUMBER	WEIGHT	SENSITIZING DOSE 0.1 PER CENT ALBUMEN		DESENSITIZING DOSE MILK PROTEIN		SHOCKING DOSE 0.1 PER CENT ALBUMEN		RESULTS
		Date	Amount	Date	Amount	Date	Amount	
				Control pigs				
	grams	*1928*	*cc.*	*1928*	*cc.*	*1928*	*cc.*	
22	315	October 17	1.0	October 24 October 26 October 30	0 0 0	October 31	1.0	Sudden death in 3–5 minutes
23	380	October 17	1.0	October 24 October 26 October 30	0 0 0	October 31	1.0	Sudden death in 3–5 minutes
24	285	October 17	1.0	October 24 October 26 October 30	0 0 0	October 31	1.0	Sudden death in 3–5 minutes
25	315	October 17	1.0	October 24 October 26 October 30	0 0 0	October 31	1.0	Sudden death in 3–5 minutes
26	265	October 17	1.0	October 24 October 26 October 30	0 0 0	October 31	1.0	Sudden death in 3–5 minutes
27	255	October 17	1.0	October 24 October 26 October 30	0 0 0	October 31	1.0	Sudden death in 3–5 minutes
28	325	October 17	1.0	October 24 October 26 October 30	0 0 0	October 31	1.0	Sudden death in 3–5 minutes
29	305	October 17	1.0	October 24 October 26 October 30	0 0 0	October 31	1.0	Sudden death in 3–5 minutes
30	310	October 17	1.0	October 24 October 26 October 30	0 0 0	October 31	1.0	Acute shock, recovery

TABLE II

A summary of the experiments on all guinea-pigs receiving only milk protein intramuscularly and the control animals

The period between sensitizing dose and shocking dose was always two weeks.

SET NUMBER	SENSITIZING DOSE, ALL PIGS		DESENSITIZING DOSE MILK PROTEIN			SHOCKING DOSE, ALL PIGS		MILK PROTEIN					CONTROLS				
	Dilution	Amount	Injections		Days before shocking dose	Dilution	Amount	Total number of pigs	Lived		Died		Total number of pigs	Lived		Died	
			Number	Amount					M	A	S	D		M	A	S	D
		cc.		*cc.*			*cc.*										
I	1:4*	1.0	1	1.0	1	1:4	0.65	6	2	2	2	0	3	0	1	2	0
II	1:4	1.0	1	1.0	1	1:4	1.0	6	1	1	4	0	1	0	0	1	0
III	1:4	1.0	1	1.0	1	1:4	1.0	6	1	1	4	0	6	0	0	6	0
IV	1:4	1.0	1	1.0	1	1:4	1.5	6	5	0	1	0	5	0	1	4	0
V	1:100†	0.75	1	1.0	1	1:100	0.6	1	0	0	1	0	1	0	0	0	1
							1.0	9	3	0	6	0	11	0	0	9	2
VIII	1:1000	1.0	1	1.0	1	1:100	1.0	8	2	1	3	2	5	3	1	1	0
IX	1:1000	1.0	1	1.0	1	1:100	1.0	8	4	1	3	0	12	4	3	5	0
X	1:1000	1.0	2	1.0	4, 2	1:1000	1.0	7	1	1	5	1	11	0	0	10	1
XI	1:1000	1.0	2	1.0	5, 3	1:1000	1.0	7	0	0	7	0	11	0	0	11	0
XII	1:1000	1.0	2	1.0	4, 2	1:1000	1.0	7	1	0	6	0	8	1	0	7	0
XIII	1:1000	1.0	3	1.0	7, 4, 2	1:1000	1.0	6	0	0	6	0	9	1	0	8	0
XIV	1:1000	1.0	3	1.0	7, 5, 1	1:1000	1.0	7	4	0	3	0	9	0	1	8	0
XV	1:1000	1.0	3	1.0	7, 4, 1	1:1000	1.0	7	0	0	7	0	9	1	0	8	0
XVI	1:1000	1.0	4	1.0	14, 12, 9, 7	1:500	0.75	11	3	2	6	0	12	0	2	10	0
XVIII	1:1000	1.0	4	1.0	14, 12, 9, 7	1:1000	0.50	4	4	0	0	0	9	0	7	2	0
							0.75	5	4	0	1	0	0	0	0	0	0
XIX	1:1000	1.0	7	1.0	14, 12, 9, 7, 5, 3, 1	1:1000	1.0	10	8	0	2	0	10	0	0	10	0

M = none to moderate shock; A = acute shock; S = sudden death; D = delayed death.

* Fresh egg albumen.

† Purified albumen.

TABLE III

A summary of the experiments on all guinea-pigs receiving mercurochrome intravenously and the control animals

The period between sensitizing dose and shocking dose was always two weeks.

SET NUMBER	SENSITIZING DOSE, ALL PIGS		DESENSITIZING DOSE MERCUROCHROME			SHOCKING DOSE, ALL PIGS		MERCUROCHROME					CONTROLS				
	Dilution	Amount	Injections		Days before shocking dose	Dilution	Amount	Total number of pigs	Lived		Died		Total number of pigs	Lived		Died	
			Number	Amount					M	A	S	D		M	A	S	D
		cc.		*mgm. per kgm. pig*			*cc.*										
I	1:4*	1.0	1	5	1	1:4	0.65	6	4	1	1	0	3	0	1	2	0
II	1:4	1.0	1	5	1	1:4	0.7	6	5	0	1	0	1	0	0	1	0
III	1:4	1.0	1	5	1	1:4	1.0	6	1	1	4	0	6	0	0	6	0
IV	1:4	1.0	1	5	1	1:4	1.5	6	1	0	5	0	5	0	1	4	0
VI	1:1000†	0.75	1	20	1	1:1000	0.75	11	8	0	2	1	6	1	0	5	0
VIII	1:1000	1.0	1	5	1	1:100	1.0	8	2	1	5	0	5	3	1	1	0
IX	1:1000	1.0	1	5	1	1:100	1.0	9	6	0	3	0	12	4	3	5	0
X	1:1000	1.0	2	5, 10	4, 2	1:1000	1.0	6	2	1	2	1	11	0	0	10	1
XI	1:1000	1.0	2	5, 10	5, 3	1:1000	1.0	7	1	0	5	1	11	0	0	11	0
XII	1:1000	1.0	2	5, 10	4, 2	1:1000	1.0	7	1	1	5	0	8	1	0	7	0
XIII	1:1000	1.0	3	5, 10, 15	7, 4, 2	1:1000	1.0	6	0	0	6	0	9	1	0	8	0
XIV	1:1000	1.0	3	5, 10, 15	7, 5, 1	1:1000	1.0	7	2	1	4	0	9	0	1	8	0
XV	1:1000	1.0	3	5, 10, 15	7, 4, 2	1:100	1.0	7	2	0	5	0	9	1	0	8	0
XVI	1:1000	1.0	4	5, 10, 15, 20	14, 12, 9, 7	1:500	0.75	12	1	1	10	0	12	0	2	10	0

M = none to moderate shock; A = acute shock; S = sudden death; D = delayed death.

* Fresh egg albumen.

† Purified albumen.

TABLE IV

A summary of the experiments on all guinea-pigs receiving both milk protein intramuscularly and mercurochrome intravenously, and the control animals

The period between sensitizing dose and shocking dose was always two weeks.

SET NUMBER	SENSITIZING DOSE, ALL PIGS		DESENSITIZING DOSE MILK PROTEIN AND MERCUROCHROME				SHOCKING DOSE, ALL PIGS		MILK PROTEIN AND MERCUROCHROME					CONTROLS				
			Injections			Days before shocking dose			Total number of pigs	Lived		Died		Total number of pigs	Lived		Died	
	Dilution	Amount	Milk protein	Number	Mercurochrome		Dilution	Amount		M	A	S	D		M	A	S	D
		cc.	*cc.*		*mgm. per kgm. pig*			*cc.*										
I	1:4*	1.0	1.0	1	5	1	1:4	0.65	6	0	1	5	0	3	0	1	2	0
II	1:4	1.0	1.0	1	5	1	1:4	1.0	4	1	1	2	0	1	0	0	1	0
III	1:4	1.0	1.0	1	5	1	1:4	1.0	6	0	0	5	1	6	0	0	6	0
IV	1:4	1.0	1.0	1	5	1	1:4	1.5	6	1	0	5	0	5	0	1	4	0
VII	1:1000†	0.75	1.0	1	20	1	1:200	0.3	8	2	1	2	3	8	0	2	6	0
VIII	1:1000	1.0	1.0	1	5	1	1:100	1.0	8	0	1	7	0	5	3	1	1	0
IX	1:1000	1.0	1.0	1	5	1	1:100	1.0	7	2	2	3	0	12	4	3	5	0
X	1:1000	1.0	1.0, 1.0	2	5, 10	4, 2	1:1000	1.0	7	1	1	5	0	11	0	0	10	1
XI	1:1000	1.0	1.0, 1.0	2	5, 10	5, 3	1:1000	1.0	7	0	0	7	0	11	0	0	11	
XII	1:1000	1.0	1.0, 1.0	2	5, 10	4, 2	1:1000	1.0	7	1	0	6	0	8	1	0	7	0
XIII	1:1000	1.0	1.0, 1.0, 1.0	3	5, 10, 15	7, 4, 2	1:1000	1.0	7	1	0	6	0	9	1	0	8	0
XIV	1:1000	1.0	1.0, 1.0, 1.0	3	5, 10, 15	7, 5, 1	1:1000	1.0	7	2	0	5	0	9	0	1	8	0
XV	1:1000	1.0	1.0, 1.0, 1.0	3	5, 10, 15	7, 4, 2	1:1000	1.0	7	2	0	5	0	9	1	0	8	0
XVI	1:1000	1.0	1.0, 1.0, 1.0, 1.0	4	5, 10, 15, 20	14, 12, 9, 7	1:500	0.75	12	4	2	6	0	12	0	2	10	0

M = none to moderate shock; A = acute shock; S = sudden death; D = delayed death.

* Fresh egg albumen.

† Purified albumen.

1. Mercurochrome was given intravenously in a one-per-cent solution, the dosage being 5 mgm. per kilogram. When more than one injection was given, the dosage was increased 5 mgm. per kilogram

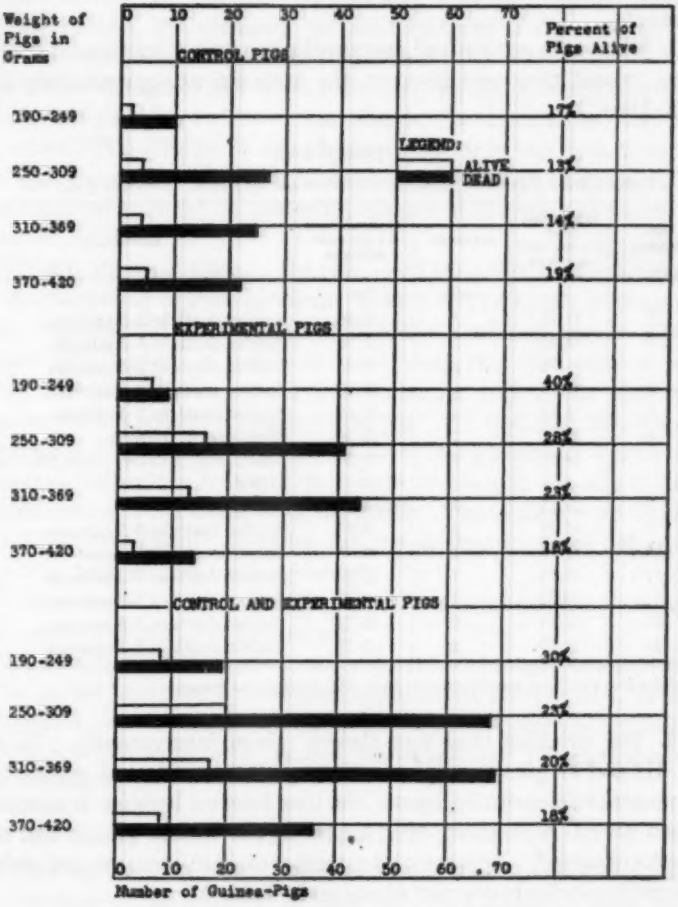

FIG. I. RELATIONS OF THE WEIGHTS OF THE SENSITIZED GUINEA PIG TO THE PROBABILITY OF ITS LIVING AFTER A SHOCKING DOSE OF ANTIGEN

Sets III, IV, V, VII, X, XI, XII, XIII, and XVI

with each successive injection. Intravenous injections were made possible by exposing neck veins by incision and injecting the mercurochrome with a ½ inch 27 gauge needle. Ligation was seldom necessary.

2. Milk protein was always given intramuscularly and in 1.0 cc. amounts.

3. When milk protein and mercurochrome were administered to the same animal, they were given on the same day at approximately the same time.

TABLE V

Typical protocol for determination of effective shocking dose. Controls of Set VI

PIG NUMBER	DESENSITIZING DOSE 0.1 PER CENT ALBUMEN	INTERVAL	SHOCKING DOSE 0.1 PER CENT ALBUMEN	RESULT
	cc.	*weeks*	*cc.*	
1	0.75	2	0.50	Sudden death in 3–5 minutes
2	0.75	2	0.50	Sudden death in 3–5 minutes
3	0.75	2	0.25	Sudden death in 3–5 minutes
4	0.75	2	0.25	Sudden death in 3–5 minutes
5	0.75	2	0.10	Sudden death in 3–5 minutes
6	0.75	2	0.50	Slight shock, recovery
7	0.75	2	0.50	Shock with recovery, but died next day
8	0.75	2	Missed vein	Not used
9	0.75	2	0.75	Sudden death in 3–5 minutes
10	0.75	2	0.75	Sub-acute shock, recovery
11	0.75	2	0.75	Sudden death in 3–5 minutes
12	0.75	2	0.75	Sudden death in 3–5 minutes
13	0.75	2	0.75	Sudden death in 3–5 minutes
14	0.75	2	0.75	Sudden death in 3–5 minutes

Effective shocking dose taken as 0.75 cc. of 0.1 per cent albumen.

4. The shocking dose was always given intravenously. Tables II, III and IV give a complete summary of the work, and specify the amounts of desensitizing agents, the time interval between treatments when several were given, the number in the various groups and the results obtained. Because of the number of experiments we are giving the protocol of only one part of one set in Table I.

B. Control animals. We have gone at length into the matter of controls in laying out our protocols because we felt it would be almost impossible to include too many animals in this group. In each

instance we have used the same number or even more controls than treated animals. In each set the effective shocking dose has been determined and all the animals (10 to 30) have been injected with this dose. We felt that the effective shocking dose need not necessarily kill all controls, but certainly the great majority of them should die and the remainder should be heavily shocked.

As to the changes of season, we found that the effective shocking dose which in September (when we first started the experiments) was 1.0 cc. of a 1:1000 dilution of egg-albumen, had to be cut to 0.75 cc. in the spring months. This change with season has often been reported. For a typical protocol for the determination of the effective shocking dose see Table V.

We have shown in Figure 1 the relation of the weights of the animals to the probability of their survival. We did this with control animals, with experimental pigs and then with the combined groups. The lighter pig received a relatively larger dose than the heavier pig because the size of the second dose of antigen varied only with the season and not with the weight of the pig. We find that the lighter the pig the greater the probability it has of living and that there is no apparent connection between the weight of the pig, the size of the second dose and the intensity of the shock. Consequently we do not feel that there is any necessity for the estimation of an effective shocking dose per grams of body-weight.

Following the injection of milk protein we were unable to see any untoward effect in any of the animals.

One rather important point which should be brought out is, that as far as the interpretation of these experiments is concerned, we do not believe that there is much difference between anaphylactic death and very acute shock with marked prostration and subsequent long delayed recovery.

CLASSIFICATION OF SHOCK

Following the intravenous injection of the effective shocking dose the reactions of the recipient animals have been classified under two main headings—'Alive' or 'Dead.'

Under 'Alive' we have two subclassifications:

a. Acute shock which may include part or all of such symptoms as—

TABLE VI

Summary of Tables II, III, and IV showing the numbers of test and control animals in the various classified reactions

a. None to moderate shock. Designated as M.
b. Acute shock. Designated as A.
c. Death—sudden or delayed. Designated as S.

NUMBER OF DESENSITIZING INJECTIONS	DAYS BEFORE SHOCKING DOSE	TOTAL NUMBER OF PIGS		M	A	S	TOTAL NUMBER OF PIGS		M	A	S	TOTAL NUMBER OF PIGS		M	A	S
1	1	34	Milk	12	4	18	35	M-220	19	2	14	30	Milk and M-220	4	3	23
		27	Control	0	2	25	21	Control	1	2	18	23	Control	0	4	19
3	7, 5, 1	7	Milk	4	0	3	7	M-220	2	1	4	7	Milk and M-220	2	0	5
		19	Control	0	1	18	19	Control	0	1	18	19	Control	0	1	18
2	5, 3, 4, 2	21	Milk	2	1	18	20	M-220	4	2	14	21	Milk and M-220	2	1	18
		30	Control	1	0	29	30	Control	1	0	29	30	Control	1	0	29
3	7, 4, 2	13	Milk	0	0	13	13	M-220	2	0	11	14	Milk and M-220	3	0	11
		18	Control	0	2	16	18	Control	0	2	16	18	Control	0	2	16
4	14, 12, 9, 7	18	Milk	11	0	7	12	M-220	1	1	10	12	Milk and M-220	4	2	6
		21	Control	0	9	12	12	Control	0	2	10	12	Control	0	2	10
7	14, 12, 9, 7, 5, 3, 1	10	Milk	8	0	2										
		10	Control	0	0	10										

TABLE VII

Summary of Tables II, III, and IV showing the percentage of guinea-pigs alive and having no to moderate shock following various methods of non-specific desensitization

NUMBER OF DESENSITIZING INJECTIONS	DAYS BEFORE SHOCKING DOSE	PERCENTAGE OF GUINEA-PIGS					
		Milk	Control	Mercurochrome	Control	Milk and mercurochrome	Control
1	1	35	0	54	4	13.3	0
3	7, 5, 1	59.1	0	28.5	0	28.5	0
2	5, 3, 4, 2	9.5	3.3	20	3.3	9.5	3.3
3	7, 4, 2	0	0	15.4	0	21.4	0
4	14, 12, 9, 7	61	0	8	0	33.3	0
7	14, 12, 9, 7, 5, 3, 1	80	0				

TABLE VIII

Summary of Tables II, III, and IV showing the percentage of guinea pigs alive and having acute shock following various methods of non-specific desensitization

NUMBER OF DESENSITIZING INJECTIONS	DAYS BEFORE SHOCKING DOSE	PERCENTAGE OF GUINEA-PIGS					
		Milk	Control	Mercurochrome	Control	Milk and mercurochrome	Control
1	1	11.8	7.4	6	10	16	17.4
3	7, 5, 1	0	5.2	12.4	5.2	0	5.2
2	5 , 3, 4, 2	4.8	0	10	0	4.8	0
3	7, 4, 2	0	11.2	0	11.2	0	11.2
4	14, 12, 9, 7	0	42.9	7.7	16.7	15.7	15.7
7	14, 12, 9, 7, 5, 3, 1	0	0				

TABLE IX

Summary of Tables II, III, and IV showing the percentage of guinea-pigs dead following various methods of non-specific desensitization

NUMBER OF DESENSITIZING INJECTIONS	DAYS BEFORE SHOCKING DOSE	PERCENTAGE OF GUINEA-PIGS					
		Milk	Control	Mercurochrome	Control	Milk and mercurochrome	Control
1	1	53.2	92.6	40	85.7	70.7	82.6
3	7, 5, 1	42.9	94.8	59.1	94.8	71.6	94.8
2	5, 3, 4, 2	85.7	96.6	70	96.6	85.7	96.6
3	7, 4, 2	100	88.8	84.6	88.8	78.6	88.8
4	14, 12, 9, 7	38.9	57.1	83.3	83.3	50	83.3
7	14, 12, 9, 7, 5, 3, 1	20	100				

coughing, sneezing, dyspnea, tachycardia, ruffling of hair, prostration, drop in rectal temperature, and rarely bucking, for these animals usually died.

b. Moderate shock, a few coughs, an anxious appearance, rubbing of nose with paws, slight or more evident dyspnea.

Under 'Dead' there are also two sub-classifications:

a. Sudden death which means exitus in three to five minutes preceded by symptoms listed above under acute shock.

b. Delayed death—where exitus is preceded by a prolonged period (5 to 60 minutes) of marked prostration as well as the other symptoms of acute shock.

An analysis of the results can much better be shown by an examination of Table VI than by citing a list of figures and percentages. The results are also grouped under three headings as percentages to describe the type of reaction which the test animal showed after receiving the effective shocking dose. See Tables VII, VIII and IX. The headings are:

a. Animals which showed none or only moderate shock.

b. Animals which showed acute shock.

c. Animals which showed severe shock and died.

We were obliged to omit entirely from our records of results Sets VIII and IX because we were unable to obtain an effective shocking dose on enough controls to warrant an interpretation.

DISCUSSION

We see first that mercurochrome exhibits a protective action when given the day before and when given 7, 5 and 1 days before the injection of the shocking dose. Milk protein and the combination of mercurochrome and milk protein have this same value. When the substances were given during the first week of sensitization, mercurochrome had no effect whatever; considerable effect was obtained from milk protein, and less from mercurochrome and milk protein together.

The greatest protection was obtained in the group in which milk protein was injected into the animals every other day for two weeks and the shocking dose was given the day following the last injection.

The results indicate that the animals can be protected against one

effective shocking dose, although in Set XVIII there is fair evidence that they survived two to three times that amount.

CONCLUSIONS

Mercurochrome and milk protein, if given at a certain time before the shocking dose, will protect a definite percentage of the animals against anaphylactic death when the amount of antigen injected is about the effective shocking dose.

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II. DEVELOPMENT OF SKIN SENSITIZATION IN GUINEA-PIGS IN RESPONSE TO INTRADERMAL INJECTIONS OF BACTERIA

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In connection with some work which we have been carrying out in regard to non-specific desensitization we wished to determine: (*a*) Whether it was possible to sensitize the skin of guinea-pigs with bacteria other than tubercle bacilli when primarily injected intradermally. (*b*) How long it would take to produce this sensitization. (*c*) Approximately how long this sensitization would last.

Consequently we instituted the method outlined below and were able to demonstrate the phenomenon which we believe to be a new observation.[1]

Mackenzie and Woo (1) in 1925 injected guinea-pigs intracutaneously with an alkaline extract of pneumococci. After several successive daily injections the pigs developed an allergic reaction in the skin in about 20 to 22 days. This reaction was temporary.

That same year A. R. Dochez and L. Sherman and also H. Zinsser and F. B. Grinnell (2) showed that skin sensitization of the guinea-pig can be accomplished by intraperitoneal, subcutaneous and intracutaneous injections of streptococci, and that subsequent skin sensitiveness can be obtained with various streptococcus materials. Their reactions occurred in 7 to 21 days.

Andrews, Derick and Swift (3) in 1926 reported some observations on a remarkable phenomenon:

[1] After this paper had been presented there appeared in the literature a most interesting contribution by C. G. Bull and C. M. McKee in the American Journal of Hygiene, 1929, **9**, 666, "The Sensitization of Rabbits to the Products of the Pneumococcus Resulting from an Acute Infection with this Organism." Therein they show that they were able to demonstrate skin sensitization in rabbits to the pneumococcus 48 hours after they were infected with intranasal instillations of the virulent bacteria. The reactions that they obtained were quite severe and their work is corroborative of ours.

Rabbits inoculated intradermally with certain strains of green streptococci have well marked lesions which, after reaching a maximum size in 24 to 48 hours and then beginning to retrogress, show in over 50 per cent of the animals a secondary increase in size and other signs of inflammation about 8 or 9 days after inoculation. [They could not produce this type of "delayed reaction" in guinea-pigs similarly treated.]

A second inoculation of green streptococci into a rabbit within 9 days of the first injection is followed by a primary but not by a secondary reaction. This inhibition of the secondary reaction is not specific; for the phenomenon of secondary reaction can be completely inhibited by previous inoculations of the rabbit with any living streptococci and usually with pneumococci. Killed organisms are usually less effectual. |

Zinsser and Grinnell (4) in 1927 reported some extremely interesting observations on guinea-pigs intradermally injected with autolysate of pneumococci. They showed that

sensitization may be brought about with extraordinary speed; originally negative animals becoming moderately positive within five, six or seven days under the influence of daily injections of 0.1 to 0.2 of the autolysates. The reaction resembles the tuberculin reaction, not only in severity and general character, but in the fact of the heat stability of the antigen, the difficulty of passive sensitizations and the difficulty of neutralization by the sera of sensitized or immunized animals.

We obtained not only an oedematous swelling of considerable size, but there is often central hemorrhage and not infrequently necrosis that leads to ulceration.

In 1927 Derick and Swift (5) continued the work begun two years before. Here they were enabled to show another interesting phenomenon:

Skin reaction: Inoculation of a rabbit, showing a secondary reaction, with a small dose of culture, i.e., 0.0001 cc., is followed by a local lesion much more intense and of distinctly longer duration than that seen in a normal rabbit. This lesion often has a dull red color comparable with that seen in a nodular syphilide or in lupus nodules. Re-inoculation of the skin of a secondary reacting rabbit with larger doses is followed by marked oedema and other signs of a more intense reaction than are shown by normal rabbits inoculated with similar amounts. These increasingly severe skin reactions are very comparable with local tuberculin reactions in man, or with the response to cutaneous inoculation with tubercle bacilli in tuberculous animals.

As their animals did not develop the secondary reaction for 8 to 9 days the second inoculation must have been given at this time. This

shows that a condition of "hyperergy" has developed in rabbits at this time against certain strains of non-hemolytic streptococci.

Julianelle and Avery (6) in 1928 say,

> A study of the development of hypersensitiveness in animals following experimental infection is in progress. The evidence at present indicates that rabbits surviving a slowly progressive but localized infection in the skin become hypersensitive to pneumococcus protein and that this state may appear as early as 12 days after the onset of the lesion.

Bull and McKee (7) have shown that rabbits which have recovered from an intranasal inoculation of virulent pneumococci are skin-sensitive to the autolysate of pneumococci.

Quite recently in this year, 1929, Hitchcock and Swift (8) have shown that a considerable number of indifferent streptococci "isolated from patients suffering from active rheumatic fever, others from persons in sound health who have never experienced disease; still others from hospital patients suffering from conditions unrelated to rheumatism," have the ability to develop secondary reactions in rabbits that received intracutaneous inoculations.

METHOD

Thirty-six guinea-pigs were used in the first series. The animals were divided into six sets. Each set received the same amount, 0.2 cc., of the bacteria listed below and this amount was always given intradermally:

Set I. Virulent pneumococcus type I.
Set II. Virulent pneumococcus type I heated 30° minutes at 60°C.
Set III. Streptococcus hemolyticus.
Set IV. Streptococcus hemolyticus heated 30 minutes at 60°C.
Set V. Staphylococcus aureus 209.
Set VI. Staphylococcus aureus 209 heated 30 minutes at 60°C.

The emulsions were made with 3 cc. of saline washings of two blood-agar slants for each set. The 0.2 cc. of pneumococcus, given intraperitoneally, killed white mice in 24 to 48 hours.

The subsequent emulsions which were used for determination of skin sensitization were made as for the initial inoculation but were always heated for 30 minutes at 60°. The emulsions were freshly

made for the first experiment. For the others a larger amount was made and allowed to stand in the ice-box for several days. All tests were made with 0.2 cc. amounts and given intradermally.

These injections were made for a period of two weeks at daily intervals. On the first day all the animals were injected. Subsequently, daily tests were made but only upon two animals out of each set. Therefore, the test was repeated every fourth day on the same animal. In this way we hoped to avoid making reactions with the constant repetition of the tests on the same animals daily. A new area was always selected and at some distance from the preceding one.

OBSERVATIONS

I. Initial inoculation

At the site of the injection there appeared in 10 to 18 hours a small firm area of induration about 0.4 cm. in diameter, which only occasionally showed any inflammation. There was never necrosis or ulcer formation. The local lesion produced by the heated emulsion was practically the same as that produced by the living bacteria; the induration tended to disappear sooner. In no case did further injection of antigen effect any change in the gradual disappearance of the initial lesion.

II. Skin response to subsequent injections

One day (24 hours) after the primary inoculation, the animals were all injected with the heated emulsions. In no pig was the slightest response demonstrated.

Two days (48 hours) later, a second intradermal test was made in two animals of each set. Within 10 hours each one of the animals responded with an area of induration of about 0.8 cm. and inflammation of about 1.0 cm., which was different and more intense than that seen after the initial injection. The height of the reaction was reached in 24 hours and gradually disappeared in three to five days.

Throughout a period of two weeks the animals responded in a similar manner. At that time they were in fairly good condition although they had lost some weight. For a typical protocol of one guinea-pig see Table I.

III. Controls

Six animals were given injections of virulent pneumococci type I; 0.2 cc. of the same emulsion as that injected into the test animals. These animals were put aside for observation and they developed the same sort of lesion as described under "Initial inoculation." There was a gradual retrogression of the size of the lesion and never a "secondary reaction." At the end of a month the animals were alive but had lost weight.

TABLE I

Protocol of one guinea-pig, weighing 240 grams

DAY	INOCULATION WITH LIVING PNEUMOCOCCUS TYPE I	REINOCULATION WITH HEATED PNEUMOCOCCUS TYPE I	SKIN REACTION					
			Immediate	10 hour	24 hour	48 hour	72 hour	96 hour
	cc.	cc.		cm.	cm.	cm.	cm.	
1	0.2	0	0	0.2	0.4	0.4	Fading	Almost gone
2	0	0.2	0	0	0	0	0	0
3	0	0.2	0	0.6	1.0	1.0	Fading	Almost gone
4	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
7	0	0.2	0	0.8	1.3	1.0	Fading	Almost gone
8	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
10	0	0.2	0	1.0	1.8	1.8	1.0	Almost gone

Two other sets of six animals apiece have shown the same type of reaction to virulent pneumococcus type I. The response to the other two varieties of bacteria and to the heated bacteria was not tried. Another set was tested at intervals for a month; at the end of this period the skin's response to antigen was definitely less than it had been at the end of a week or ten days.

Three controls injected with the washings from peptone-agar culture media did not produce sensitizations. As an added control the washings from blood-agar culture media were given to three

TABLE II

Group skin response in guinea pigs to intradermal injections of heat killed antigen at various periods after an initial intradermal injection with living or heat killed organisms

MANNER OF INOCULATION		TYPE OF SKIN REACTION FOLLOWING REINJECTION WITH HEAT KILLED ANTIGEN																	
Kind of bacteria	Condition of bacteria for primary inoculation	Number of pigs	Days after initial inoculation																
			1	2	3	4	5	6	7	8	9	10	12	14	16	18	22	25	28	31
Pneumococcus Type I	Living	6	0	+	+	++	++	++	++	++	+++	+++	+++	++	++	++	+++	+	+	+
	Heated	6	0	+	+	+	++	++	++	++	+++	+++	+++							
Streptococcus hemolyticus	Living	6	0	+	+	++	++	++	++	+++	+++	+++	+++							
	Heated	6	0	+	+	++	++	++	++	++	+++	+++	+++							
Staphylococcus Aureus 209	Living	6	0	+	+	+	++	++	++	++	++	++	++							
	Heated	6	0	+	+	+	++	++	++	++	++		++							

0 = no reaction. + = induration 0.8 cm.; inflammation 1.0 cm. ++ = induration 1.0–1.5 cm., inflammation 1.5–2.0 cm. +++ = induration 2.0 cm.; inflammation 2.5 cm.

TABLE III

Group skin response in control guinea-pigs which were not reinjected after an initial intradermal injection of living or heat killed organisms

MANNER OF INOCULATION			TYPE OF SKIN REACTION FOLLOWING INITIAL INJECTION									
Kind of bacteria	Condition of bacteria for inoculation	Number of pigs	Days after initial injection									
			1	2	3	4	5	6	7	8	9	10	12
Pneumococcus Type I	Living	2	Induration 0.4 cm. Inflammation 0.5 cm.	Induration 0.4 cm.	±	0	0	0	0	0	0	0	0
	Heated	2	Induration 0.4 cm. Inflamation 0.5 cm.	Induration 0.4 cm.	±	0	0	0	0	0	0	0	0
Streptococcus hemolyticus	Living	2	Induration 0.4 cm. Inflammation 0.5 cm.	Induration 0.4 cm.	±	0	0	0	0	0	0	0	0
	Heated	2	Induration 0.4 cm. Inflammation 0.5 cm.	Induration 0.4 cm.	±	0	0	0	0	0	0	0	0
Staphylococcus Aureus 209	Living	2	Induration 0.4 cm. Inflammation 0.5 cm.	Induration 0.4 cm.	±	0	0	0	0	0	0	0	0
	Heated	2	Induration 0.4 cm. Inflammation 0.5 cm.	Induration 0.4 cm.	±	0	0	0	0	0	0	0	0

± = induration almost gone. 0 = induration gone.

animals in a manner corresponding to the bacterial emulsions controls. It was not possible to demonstrate any sensitization.

SUMMARY

Guinea-pigs injected intradermally with emulsions of whole bacteria both alive and dead (*Pneumococcus* type I, *Streptococcus hemolyticus* and *Staphylococcus aureus* 209) develop skin sensitization to the same antigen in the remarkably short period of forty-eight hours. This is, so far as we have been able to ascertain, the first time this observation has been made.

The skin sensitization remained for at least a month but by that time had diminished. The strongest sensitization was developed about ten days after the primary inoculation.

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III. MILK PROTEIN AS AN INHIBITOR OF SKIN SENSITIZATION IN GUINEA-PIGS PREVIOUSLY INJECTED INTRADERMALLY WITH PNEUMOCOCCI

LAY MARTIN, M.D., AND JUSTINA H. HILL, M.S.

In the preceding article we have shown that it is possible to demonstrate sensitization in the skin of guinea-pigs 48 hours after they have received an intradermal injection of live or dead organisms such as pneumococcus type I, *Staphylococcus aureus* and *Streptococcus hemolyticus*. This reaction is produced with regularity and increases somewhat in strength up to a week or ten days; after which time there is gradual regression.

We have shown, in No. I of this paper, that we were able to obtain partial desensitization in a certain percentage of guinea-pigs sensitized to egg-albumen by single or multiple injections of milk and mercurochrome. Therefore, it seemed reasonable to ascertain whether or not we could by intradermal and intramuscular injections of milk inhibit the development of local sensitization in guinea-pigs which had received intradermal injections of virulent pneumococci.

METHOD

The eighteen-hour growth of pneumococci on six blood-agar tubes was washed off with sterile saline solution and the total made up to 5.0 cc. Of this emulsion 0.2 cc. was injected intradermally in the left flank of each of twelve white guinea-pigs.

Set I. Immediately after injection six of the pigs received 0.2 cc. of milk protein[1] into the skin of the right flank. At the end of twenty-four hours they received milk again on the right side, but in a different spot. At the end of forty-eight hours they received on the left side, some distance from the first injection of virulent pneumococci, an emulsion of the same organism which had been heated, this time,

[1] The milk protein used was Aolan, distributed in this country by the Duke Laboratories, Inc. They have kindly kept us fully stocked.

for thirty minutes at 60°C. The amount injected was again 0.2 cc. At about the same time they received 0.2 cc. milk protein on the right side.

Set II. Six pigs were kept as controls and at the end of forty-eight hours received a 0.2 cc. injection of the heated antigen.

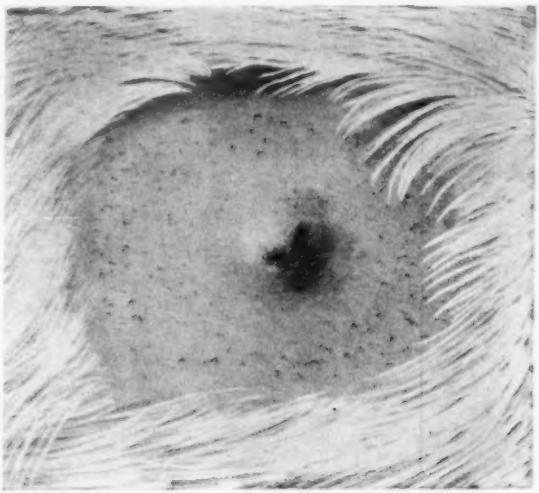

FIG. 1. CONTROL ANIMAL. SKIN RESPONSE TO REINJECTION OF HEATED PNEUMOCOCCUS TYPE I 48 HOURS AFTER INITIAL INTRADERMIC INJECTION WITH LIVING PNEUMOCOCCUS TYPE I

OBSERVATIONS

On the morning of the third day the pigs were examined. As was expected, the control animals showed an area of induration and inflammation. The six animals that received milk protein intradermally showed only the slightest response to the antigen and none to the milk protein. The difference between the two reactions is well shown in the drawings (Figs. 1 and 2).

A second series of eighteen animals was divided into three sets; the first two sets were treated as in the first series. Set III received

the milk protein in 1.0 cc. amounts, but intramuscularly; otherwise this set was treated as Set I.

The control and intradermally treated animals showed response to the bacterial antigen in the same manner as those in the first series. The six animals that received the milk protein intramuscularly showed some induration and swelling in response to the bacterial antigen, but this induration and swelling were more intense than that produced in

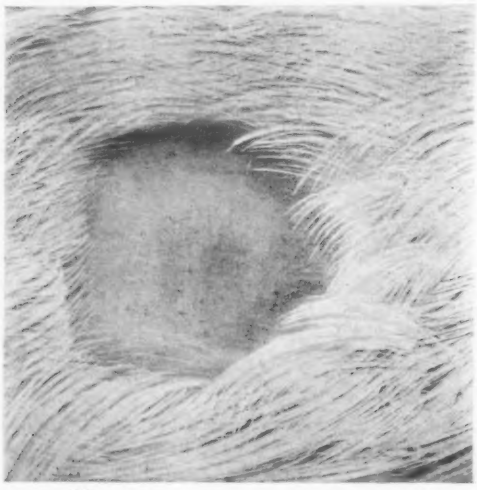

FIG. 2. TEST ANIMAL WHICH HAD RECEIVED 3 INTRADERMIC INJECTIONS OF MILK PROTEIN DURING PERIOD OF SENSITIZATION. MARKED DIMINUTION IN SKIN RESPONSE TO REINJECTION OF HEATED PNEUMOCOCCUS TYPE I 48 HOURS AFTER INITIAL INTRADERMAL INJECTION WITH LIVING PNEUMOCOCCUS TYPE I

the intracutaneously treated animals. On the other hand, the reaction throughout the set was less than that seen in the controls.

The same animals received almost daily milk injections for ten days. During this period their sensitization was tested on the second, seventh, and tenth days. The same type of response to the bacterial antigen as noted above was demonstrated on each test. The milk protein injections were always given on one side of the body, the

TABLE I

Collective grouping of animals. Skin response to intradermal injections of heat killed antigen in treated and untreated animals at various periods after an initial

TABLE I

Collective grouping of animals. Skin response to intradermal injections of heat killed antigen in treated and untreated animals at various periods after an initial intradermal injection of living bacteria

DATE	12 TEST AND 6 CONTROL ANIMALS	6 TEST ANIMALS —SET I	6 TEST ANIMALS —SET III	6 CONTROL ANIMALS —SET II	SKIN REACTIONS TO LIVING PNEUMOCOCCUS TYPE I (PRIMARY INJECTION)			SKIN REACTIONS TO HEATED PNEUMOCOCCUS TYPE I (REINJECTIONS)			SKIN REACTIONS TO MILK PROTEIN
	Pneumococcus Type I	Intradermal milk protein	Subcutaneous milk protein	Milk protein	Intradermal Set I	Subcutaneous Set III	Controls Set II	Intradermal Set I	Subcutaneous Set III	Controls Set II	Intradermal Set I
		cc.	cc.								
April 9	Living 0.2 cc.	0.2	1.0	0							
April 10	0	0.2	1.0	0	Induration about 0.4 cm. Inflammation 0 to 0.5 cm.	Induration about 0.4 cm. Inflammation 0 to 0.5 cm.	Induration about 0.4 cm. Inflammation 0 to 0.5 cm.				
April 11	Heated 0.2 cc.	0.2	1.0	0	Induration the same Inflammation gone	Induration the same Inflammation gone	Induration the same Inflammation gone				
April 12	0	0.2	1.0	0	Almost gone	Almost gone	Almost gone	Induration about 0.4 cm. Inflammation 0 to 0.5 cm.	Induration about 0.8 cm. Inflammation about 1.0 cm.	Induration about 1.0 cm. Inflammation 1.0 to 2.0 cm.	
April 13	0	0.2	1.0	0	None	None	None	Practically gone	About the same	About the same	
April 14	0	0	0	0				None	Almost gone	Induration 0.5 to 0.8 cm. Inflammation about 1.0 cm.	
April 15	0	0.2	1.0	0				None	None	Almost gone	
April 16	Heated 0.2 cc.	0.2	1.0	0				None	None	Almost gone	Induration about 0.8 cm Inflammation 1.5 to 2.0 cm.
April 17	0	0.2	1.0	0				Induration about 0.4 cm. Inflammation 0 to 0.5 cm.	Induration about 0.8 cm. Inflammation about 1.0 cm.	Induration 1.5 to 2.0 cm. Inflammation 1.5 to 2.5 cm.	About the same
April 18	0	0.2	1.0	0				Almost gone	Induration about 0.6 cm. Inflammation about 0.8 cm.	About the same	About the same
April 19	Heated 0.2 cc.	0.2	0	0				None	Almost gone	Induration about 1.0 cm. Inflammation about 1.5 cm.	About the same
April 20	0	0	0	0				Induration about 0.4 cm. Inflammation 0 to 0.5 cm.	Induration about 0.8 cm. Inflammation about 1.0 cm.	Induration 1.5 to 2.0 cm. Inflammation 1.5 to 2.5 cm.	
April 22	0	0	0	0				None	About the same	About the same	

antigen on the other. Care was taken to keep the respective injections as far as possible from the preceding ones. The chart will give detailed information.

Skin sensitization to milk protein was demonstrated at the end of a week during which time they had received daily intradermal injections of milk protein. In this reaction the swelling and inflammation were milder than in bacterial antigen response.

SUMMARY.

Those animals which received daily intradermal injections of milk protein were markedly less sensitive to a second injection of bacterial antigen than the controls.

Intramuscular injection of milk protein was less effective in inhibiting hypersensitivity to the bacterial antigen.